

## Phenazepam - a new drug of abuse in Finland – findings from apprehended drivers

Lars Wilhelm<sup>1</sup>, Stefan Jenckel<sup>1</sup>, Pirkko Kriikku<sup>2</sup>, Janne Rintatalo<sup>3</sup>, Jukka Hurme<sup>2</sup>, Jan Kramer<sup>1,4</sup>

<sup>1</sup> LADR MVZ Dr. Kramer und Kollegen, Lauenburger Str. 65, D-21502 Geesthacht, Germany

<sup>2</sup> Vita Health Care Services Ltd., Vita Laboratory, Laivakatu 5 F, FIN-00150 Helsinki, Finland

<sup>3</sup> National Bureau of Investigation Forensic Laboratory, Jokiniemenkuja 4, FIN-01370 Vantaa, Finland

<sup>4</sup> Medical Department I, University of Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany

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### Abstract

**Aim:** Phenazepam is a benzodiazepine that has not been approved for prescription use in Finland. However, it is seen considerably often in blood samples from drivers apprehended for driving under the influence of drugs (DUID) in Finland. Some of the positive cases might be from Russian drivers, since in Russia phenazepam is used e.g. to treat epilepsy. The majority of our findings, however, are likely to result from illegal use. We report the findings from a half year period in 2010 from DUID suspects in Finland.

**Methods:** For the analysis of phenazepam from serum or plasma samples an LC-MS/MS system (AB Sciex API 4000) is used. The sample preparation includes solid phase extraction with Agilent Bond Elut Plexa cartridges. The HPLC-system is equipped with a phenyl-hexyl column from Phenomenex. MS-detection is done in positive ESI mode in multiple reaction monitoring (MRM).

**Results and Discussion:** Case reports were reviewed to evaluate the correlation between blood concentrations and possible driving impairment. Some of the suspects underwent a clinical examination by a physician while under arrest. In phenazepam positive cases the data obtained from the clinical examination was evaluated together with the information about possible other intoxicating substances detected from the suspects blood. The aim of this evaluation was to assess the significance of phenazepam findings in DUID cases. Our data demonstrate increasing illegal use of phenazepam in Finland. Therefore, we suggest that phenazepam should be routinely included in the analysis of samples from DUI suspects.

### 1. Introduction

Phenazepam (Fig. 1) is a benzodiazepine that is used as an anxiolytic, sedative-hypnotic and anti-epileptic in Russia [1]. However it has not been approved for prescription use in Finland and, unlike other benzodiazepines, it has not been scheduled as a narcotic. However, it is frequently detected in blood samples in suspected DUID cases in Finland. A small proportion of the positive cases are drivers who obtained the drug legally in Russia, however, the majority of cases result from illegal use. The structure, therapeutic action and pharmacokinetics of phenazepam are similar to those of lorazepam [2]. Normal clinical dose in the treatment of anxiety is 0.5 – 2.0 mg resulting in a therapeutic plasma concentration between 0.020 – 0.060 mg/L [1]. The peak plasma concentration is attained about 4 hours after oral administration of phenazepam and the average elimination half life of the drug is about 60 hours [1].

To our knowledge there are very few data available concerning the toxicology of phenazepam. A recent case report suggested that phenazepam was at least partly responsible for the death of a 31-year old man in the USA [3].

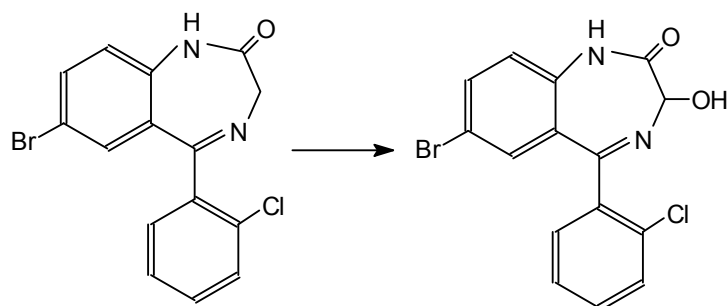


Fig. 1. Phenazepam (M=349.609 g/mol) and its metabolite 3-OH-phenazepam.

The aim of this study was to develop and validate an assay for determination of phenazepam in serum or plasma. The epidemiology of phenazepam use, and concomitant use of other drugs, in drivers suspected of driving under the influence of drugs (DUID) in Finland was investigated. The capability of phenazepam in inducing psychomotor impairment in apprehended drivers is discussed. We also report one case of phenazepam abuse in Germany.

## 2. Materials and Methods

The analysis of phenazepam in serum and plasma samples was performed on an AB Sciex LC-MS/MS API 4000 coupled with a Shimadzu LC20 HPLC.

The first step of sample preparation consisted of adding internal standard (diazepam-D5) to 0.2 mL of sample. Sample cleanup was performed by solid phase extraction (SPE) using Agilent Bond Elut Plexa cartridges. The analytes were eluted with 0.5 mL methanol/glacial acetic acid (98:2). 10  $\mu$ L of the eluate were injected into a phenyl-hexyl 50 x 3.0 mm 3  $\mu$ m column from Phenomenex and eluted with an acetonitrile/ammonium acetate buffer gradient at an oven temperature of 40°C; total run time being 6 min. The detection was done in positive ESI mode in multiple reaction monitoring (MRM) observing the following ion transitions: phenazepam  $m/z$  351/206 and  $m/z$  351/186 and the internal standard diazepam-D5  $m/z$  290/262. A 6 point calibration curve was constructed with concentrations from 0.005 to 0.1 mg/L.

The limit of detection (LOD) for phenazepam was 0.00144 mg/L and the limit of quantification (LOQ) 0.00306 mg/L. The calibration was linear over the range 0.005 - 1.000 mg/L. For sample concentrations exceeding the calibration range the curve was extended and an approximate value was given as a result. Extraction recovery and relative signal intensity were determined at two concentrations (0.050 mg/L and 0.500 mg/L) and were found to be 88.3 % and 124.3 %, respectively. Reproducibility and accuracy were examined at two concentrations (0.020 and 0.060 mg/L) measured on two occasions, 8 days apart. Reproducibility was found to be 3.7 % and accuracy was 11.36 %. Selectivity was tested by analysing six negative samples and samples spiked with common drugs (benzodiazepines, opioids, amphetamines); no interference could be detected.

LC-MS/MS chromatograms of a blank, a standard solution and a real sample are presented in Fig. 2.

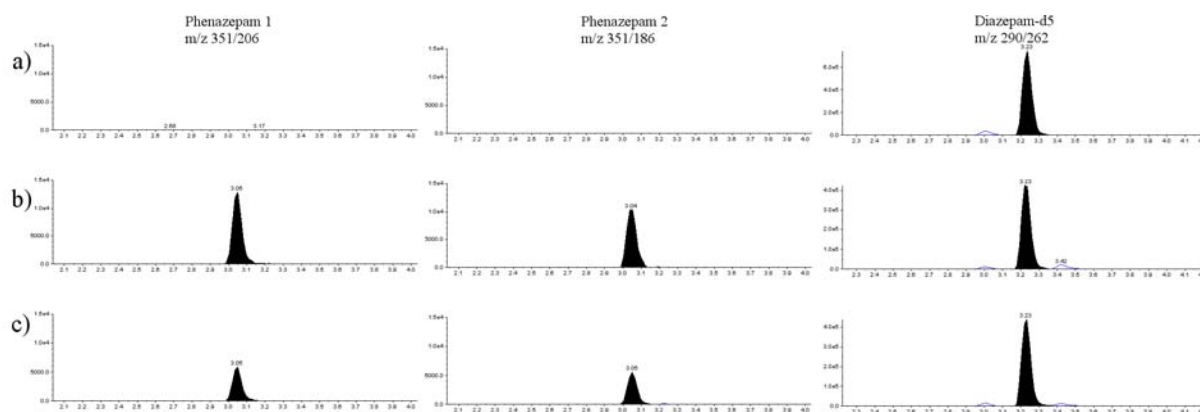


Fig. 2. Chromatograms of a) a blank b) a phenazepam standard 0.040 mg/l c) a serum sample containing 0.0174 mg/L of phenazepam.

### 3. Results and Discussion

In a half year period between July and December in 2010 a drug analysis was requested 2416 times from drivers apprehended because of the suspicion of DUID. Of these samples 83 were found to be positive for phenazepam. This represents approximately 3.4 % of all confirmed DUID cases, excluding those cases tested for alcohol exclusively. Since in 2003 there were only 20 positive phenazepam cases among DUID suspects in Finland within the whole year [2], the prevalence of phenazepam has increased significantly. This phenomenon has also been observed in Sweden [4]. The median phenazepam concentration in our study was 0.098 mg/L which is above the concentration seen at prescribed doses. The range of concentrations seen in positive samples was 0.005 – 3.000 mg/L (up to 50 times therapeutic range).

As shown in Fig. 3, other drugs of abuse or medications were present in 77 cases. In more than a third of the cases the concentration of phenazepam was at least twice the maximum of the therapeutic range. In 6 of these 83 cases no other drugs in relevant concentrations beside phenazepam were detected. Details of these 6 cases as well as of one phenazepam intoxication case from Germany are given in Table 1.

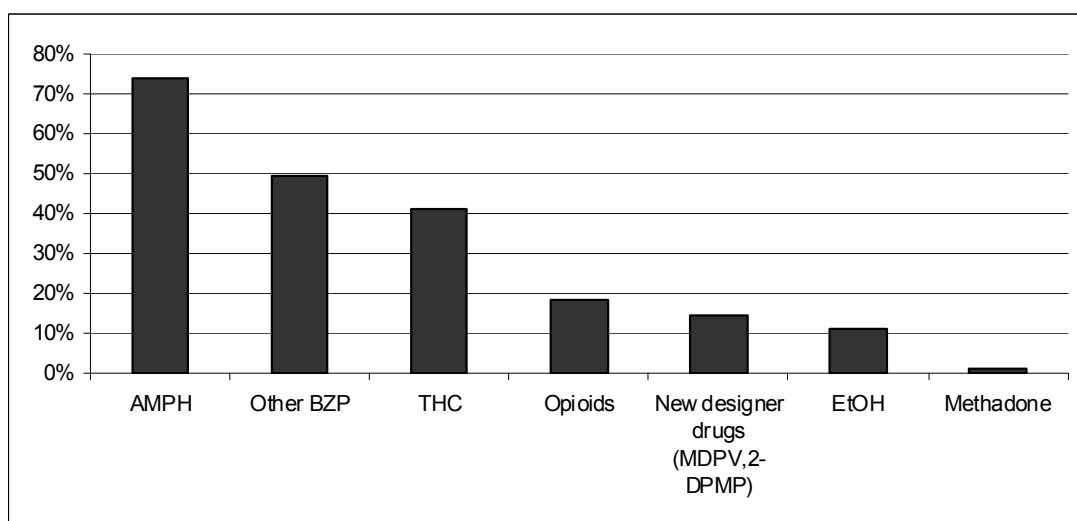


Fig. 3. Concomitant use of drugs in phenazepam positive DUID cases.

Of the 6 cases where phenazepam was considered to be the only relevant drug, a psychophysical achievement deficiency test was performed by a physician after the arrest in 4 cases.

In all of these cases the deficiency test showed aberrations and in 2 of them the aberrations were sufficient to cause functional disorder. It can be concluded that in these 4 cases the aberration/functional disorder was caused by phenazepam exclusively. Typically the aberrations included unstable gait, difficulties in determining the current time, difficulties in walking on a straight line and turning around as well as delayed pupil reaction to light.

Tab. 1. Cases in which phenazepam was implicated in psychomotor aberrations/functional disorder.

Sample	Gender	Age	Phenazepam [mg/L]	Other findings	Location	Clinical examination
1-7/2010	female	50	0.270		Helsinki area (FIN)	aberrations and moderate or greater functional disorder detected
2-7/2010	male	27	0.310	Citalopram (trace)	Helsinki area (FIN)	aberrations and mild functional disorder detected
3-7/2010	male	46	0.044	Alcohol 0.39 g/L	Eastern border district (FIN)	no data
4-7/2010	female	23	0.170	THC-COOH (trace)	Eastern border district (FIN)	no data
5-7/2010	male	21	3.000		Northern Finland (FIN)	aberrations detected, no overall functional disorder
6-7/2010	female	47	0.230		Helsinki area (FIN)	aberrations detected, no overall functional disorder
7-1/2011	male	45	0.220	Diazepam (trace) Nordiazepam: 0.0617	Hamburg (GER)	acute intoxication

The seventh case in Table 1 is a sample from northern Germany. A 45 year old man had purchased phenazepam from the internet and was later admitted to the hospital because of clinical signs of intoxication. His blood screened positive for benzodiazepines. In the confirmation analysis a blood phenazepam concentration of 0.220 mg/L was detected along with 0.0617mg/L of nordiazepam and a trace of diazepam.

#### 4. Conclusions

Phenazepam is not available legally in Finland but is being widely abused. In a 6 month period in 2010 there were 83 positive cases among apprehended drivers; other drugs were usually also present. A wide range of concentrations was found with many samples well above the therapeutic range. In a few cases with high concentrations, phenazepam was clearly the cause of psychomotor impairment. However, the possibility of tolerance makes it difficult to define levels that cause impairment. Our data demonstrate increasing illegal use of phenazepam at least in Finland. We suggest that analysis of phenazepam should be included routinely in the examination of samples from DUID suspects.

#### 5. References

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